CLAIMS

What is claimed is:

1. A method of identifying a compound which covalently binds to the surface of a target protein in sufficient proximity to the binding site between a macromolecular ligand and the target protein to inhibit binding of the macromolecular ligand with the target protein, said method comprising the steps of:

   a) selecting a lead compound which non-covalently binds to the surface of a target protein with a Kd of greater than about 0.10 μM, wherein said lead compound is represented by the structural formula T-H;

   b) preparing a plurality of analogs of the lead compound, each analog being represented by the structural formula T-L-A, wherein L is an inert linking group, A is a moiety comprising a reactive functional group and -L-A, taken together, is different for each analog;

   c) combining the target protein, macromolecular ligand and each analog under conditions suitable for binding between the target protein and macromolecular ligand;

   d) assaying each combination of step c) for inhibition of macromolecular ligand/target protein binding and for covalent binding between the analog and the target protein; and

   e) selecting analogs which inhibit macromolecular ligand/target protein binding and which covalently bind with the target protein.

2. The method of Claim 1 wherein the lead compound inhibits binding of the macromolecular ligand with the target protein.

3. The method of Claim 2 additionally comprising the steps of:

f) preparing a plurality of additional analogs of an analog selected in step e);

g) combining the target protein, macromolecular ligand and each additional analog under conditions suitable for binding between the target protein and macromolecular ligand;

h) assaying each combination of step g) for inhibition of macromolecular ligand/target protein binding and for covalent binding between the additional analog and the target protein; and

i) selecting additional analogs with improved inhibition of macromolecular ligand/target protein binding compared with the analog selected in step e).

4. The method of Claim 3 additionally comprising the step of repeating steps f)-h) with an analog selected in step i) and selecting analogs with improved inhibition of macromolecular ligand/target protein binding compared with the analog selected in step i).

5. The method of Claim 2 wherein the lead compound is selected by screening a combinatorial library of compounds for inhibition of target protein/macromolecular ligand interaction.

6. The method of Claim 2 wherein the complex between the target protein and macromolecular ligand is modeled computationally, by x-ray crystallography; by nuclear magnetic resonance spectrophotometry or by active site localization; the target protein/macromolecular ligand binding site is identified from the model(s); and wherein a lead compound is designed based on its ability to bind to the protein target/macromolecular ligand binding site.

7. The method of Claim 2 additionally comprising the steps of:

a) modeling the complex between the target protein and the lead compound computationally, by x-ray crystallography; by nuclear magnetic resonance spectrophotometry or by active site localization;

b) identifying reactive functional groups on the surface of the target protein in the vicinity of the binding site between the target protein and lead compound; and

c) selecting A groups that can form covalent bonds with the reactive functional groups on the surface of the protein; and

d) selecting L groups that will bring the A groups into sufficient proximity with the reactive functional groups on the surface of the protein to covalently react after binding between the targeting group and the target protein.

8. The method of Claim 2 wherein the reactive group has a reactivity with the corresponding free amino acid under physiological conditions of less than about 10^{-4} $M^{-1}sec^{-1}$.

9. The method of Claim 2 wherein the linking group is inert.

10. The method of Claim 2 wherein said linking group is cleavable *in vivo*.

11. The method of Claim 2 wherein the targeting group is degradable *in vivo*.

12. The method of Claim 10 or 11 wherein the compound has an *in vivo* half-life of at least about one minute.

13. The method of Claim 8 wherein the targeting group is a carbohydrate, natural product, peptide, protein, antibody or monoclonal antibody.

14. A method of identifying a compound which covalently binds to the surface of a target protein in sufficient proximity to the binding site between a macromolecular ligand and the target protein to inhibit binding of the macromolecular ligand with the target protein, said method comprising the steps of:

a) selecting a lead compound which non-covalently binds to the surface of a target protein, wherein said lead compound is represented by the structural formula T-H;

b) preparing a plurality of analogs of the lead compound, each analog being represented by the structural formula T-L-A, wherein L is a linking group, A is a moiety comprising a reactive functional group, -L-A, taken together, is different for each analog and the linking group is cleavable *in vivo* or the targeting group is degradable *in vivo*;

c) combining the target protein, macromolecular ligand and each analog under conditions suitable for binding between the target protein and macromolecular ligand;

d) assaying each combination of step c) for inhibition of macromolecular ligand/target protein binding and for covalent binding between the analog and the target protein; and

e) selecting analogs which inhibit macromolecular ligand/target protein binding and which covalently bind with the target protein.

15. The method of Claim 14, wherein the lead compound inhibits binding of the macromolecular ligand with the target protein.

16. The method of Claim 15, additionally comprising the steps of:

f) preparing a plurality of additional analogs of an analog selected in step e);

g) combining the target protein, macromolecular ligand and each additional

analog under conditions suitable for binding between the target protein and macromolecular ligand;

h) assaying each combination of step g) for inhibition of macromolecular ligand/target protein binding and for covalent binding between the additional analog and the target protein; and

i) selecting additional analogs with improved inhibition of macromolecular ligand/target protein binding compared with the analog selected in step e).

17. The method of Claim 16 additionally comprising the step of repeating steps f)-h) with an analog selected in step i) and selecting analogs with improved inhibition of macromolecular ligand/target protein binding compared with the analog selected in step i).

20. The method of Claim 15 wherein the lead compound is selected by screening a combinatorial library of compounds for inhibition of target protein/macromolecular ligand interaction.

19. The method of Claim 15 wherein the complex between the target protein and macromolecular ligand is modeled computationally or by x-ray crystallography; the target protein/macromolecular ligand site is identified from the model(s); and wherein a lead compound is designed based on its ability to bind to the protein target/macromolecular ligand binding site.

20. The method of Claim 15, additionally comprising the steps of:

a) modeling the complex between the target protein and lead compound computationally, by x-ray crystallography; by nuclear magnetic resonance spectrophotometry or by active site localization;

b) identifying reactive functional groups on the surface of the target protein

in the vicinity of the binding site between the target protein and lead compound; and

c) selecting groups that can form covalent bonds with the reactive functional groups on the surface of the protein; and

d) selecting L groups that will bring the A groups into sufficient proximity with the reactive functional groups on the surface of the protein to covalently react after binding between the targeting group and target protein.

21. The method of Claim 15 wherein the compound has an *in vivo* half-life of at least about one minute.

22. The method of Claim 15 wherein the linking group is inert.

23. The method of Claim 21 wherein the compound has a molecular weight greater than about 1500 amu.

24. The method of Claim 21 wherein the targeting group binds non-covalently to a surface of the target protein with a Kd of greater than about 0.10 μM.

25. The method of Claim 24 wherein the reactive group has a reactivity with the corresponding free amino acid under physiological conditions of less than about 10^{-4} $M^{-1}sec^{-1}$.

26. The method of Claim 21 wherein the targeting group is a carbohydrate, natural product, peptide, protein, antibody or monoclonal antibody.

27. A compound for inhibiting binding between a target protein and a macromolecular ligand of the target protein, said compound comprising a

targeting group and an attaching group, wherein:

the targeting group is a moiety that binds non-covalently to a surface of the target protein with a Kd of greater than about 0.10 μM and within sufficient proximity to the target protein/macromolecular ligand binding site such that the compound inhibits binding between the target protein and the macromolecular ligand; and

the attaching group is a moiety comprising a reactive functional group which can form a covalent bond with an amino acid on the surface of the target protein after the targeting group binds with the target protein.

28. The compound of Claim 27 wherein the reactive functional group has a reactivity with the corresponding free amino acid under physiological conditions of less than about 10^{-4} $M^{-1}sec^{-1}$.

29. The compound of Claim 27 additionally comprising an inert linking group which connects the targeting group with the attaching group.

30. The compound of Claim 27 additionally comprising a linking group which connects the targeting group with the attaching group, wherein said linking group is cleavable *in vivo*.

31. The compound of Claim 27 wherein the targeting group is degraded *in vivo*.

32. The compound of Claim 30 or 31 wherein the compound has an *in vivo* half-life of at least about one minute.

33. The compound of Claim 27 wherein the compound has a molecular weight greater than about 1500 amu.

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34. The compound of Claim 33 wherein the targeting group is a carbohydrate, natural product, peptide, protein, antibody or monoclonal antibody.

35. A compound for inhibiting binding between a target protein and a macromolecular ligand of the target protein, said compound comprising a targeting group, an attaching group and, optionally, a linking group, wherein:

the targeting group is a moiety that binds non-covalently to a surface of the target protein and within sufficient proximity to the target protein/macromolecular ligand binding site such that the compound inhibits binding between the target protein and the macromolecular ligand;

the attaching group is a moiety comprising a reactive functional group which can form a covalent bond with an amino acid on the surface of the target protein after the targeting group binds with the target protein;

the linking group connects the targeting group and the attaching group; and

the targeting group is degradable *in vivo* or the linking group is cleavable *in vivo*.

36. The compound of Claim 35 wherein the compound has an *in vivo* half-life of at least about one minute.

37. The compound of Claim 36 wherein the compound comprises an inert linking that connects the targeting group and attaching group.

38. The compound of Claim 36 wherein the compound has a molecular weight greater than about 1500 amu.

39. The compound of Claim 38 wherein the targeting group is a carbohydrate, natural product, peptide, protein, antibody or monoclonal antibody.

40. The compound of Claim 36 wherein the targeting group binds non-covalently to a surface of the target protein with a Kd of greater than about 0.10 μM.

41. The compound of Claim 40 wherein the reactive functional group has a reactivity with the corresponding free amino acid under physiological conditions of less than about 10^{-4} $M^{-1}sec^{-1}$.

42. A method of inhibiting binding between a target protein and a macromolecular ligand in a subject in need of such inhibition, said method comprising the step of administering to the subject an effective amount of a compound comprising a targeting group and an attaching group, wherein:

the targeting group is a moiety that binds non-covalently to a surface of the target protein with a Kd of greater than about 0.10 μM and within sufficient proximity to the target protein/macromolecular ligand binding site such that the compound inhibits binding between the target protein and the macromolecular ligand; and

the attaching group is a moiety comprising a reactive functional group which can form a covalent bond with an amino acid on the surface of the target protein after the target group binds with the target protein.

43. The method of Claim 42 wherein the reactive functional group has a reactivity with the corresponding free amino acid under physiological conditions of less than about 10^{-4} $M^{-1}sec^{-1}$.

44. The method of Claim 42 additionally comprising an inert linking group which connects the targeting group with the attaching group.

45. The method of Claim 42 additionally comprising a linking group which connects the targeting group with the attaching group, wherein said linking group is

cleavable *in vivo*.

46. The method of Claim 42 wherein the targeting group is degraded *in vivo*.

47. The method of Claim 45 or 46 wherein the compound has an *in vivo* half-life of at least about one minute.

48. The method of Claim 42 wherein the compound has a molecular weight greater than about 1500 amu.

49. The method of Claim 48 wherein the targeting group is a carbohydrate, natural product, peptide, protein, antibody or monoclonal antibody.

50. A method of inhibiting binding between a target protein and a macromolecular ligand in a subject in need of such inhibition, said method comprising the step of administering to the subject an effective amount of a compound comprising a targeting group and an attaching group, wherein:

the targeting group is a moiety that binds non-covalently to a surface of the target protein and within sufficient proximity to the target protein/macromolecular ligand binding site such that the compound inhibits binding between the target protein and the macromolecular ligand;

the attaching group is a moiety comprising a reactive functional group which can form a covalent bond with an amino acid on the surface of the target protein after the targeting group binds with the target protein;

the linking group connects the targeting group and the attaching group; and

the targeting group is degradable *in vivo* or the linking group is cleavable *in vivo*.

51. The method of Claim 50 wherein the compound has an *in vivo* half-life of at least about one minute.

52. The method of Claim 51 wherein the compound comprises an inert linking group that connects the targeting group and the attaching group.

53. The compound of Claim 51 wherein the compound has a molecular weight greater than about 1500 amu.

54. The method of Claim 53 wherein the targeting group is a carbohydrate, natural product, peptide, protein, antibody or monoclonal antibody.

55. The method of Claim 51 wherein the targeting group binds non-covalently to a surface of the target protein with a Kd of greater than about 0.10 μM.

56. The method of Claim 55 wherein the reactive functional group has a reactivity with the corresponding free amino acid under physiological conditions of less than about 10^{-4} $M^{-1}sec^{-1}$.

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